OBSERVATIONS ON THE FINE STRUCTURE OF THE HORNY LAYER IN THE NORMAL HUMAN EPIDERMIS*

ISSER BRODY, M.D.

In the stratum corneum of the normal human epidermis three sub-layers are discerned: a basal, an intermediate and a superficial layer (1). The cytoplasm in the basal layer is dense throughout the cell. At the displacement of the cell from the basal to the intermediate and superficial layers the cytoplasm in its entirety shows certain well-defined changes. The fibrils display an altered affinity to uranyl acetate, and, in addition, electron-optically empty spaces appear between the keratin fibrils in some of the cells (1).

These electron-optically empty spaces, when present, give a characteristic appearance to the cells of the intermediate and superficial layers and distinguish them clearly from cells lacking these features. This investigation was carried out with the aim of ascertaining whether this distinction between two types of cells could be traced already in the basal layer.

MATERIAL AND METHODS

Human skin from the region of the shoulder was obtained from adult individuals of both sexes without any evidence of skin disease. The specimens were fixed for four hours in a 1 per cent osmium tetroxide solution at pH 7.2–7.4 (2) made isotonic to blood (3, 4). The temperature was 3–5° C during fixation, rinsing in Tyrode's solution and dehydration to the stage of 95 per cent ethyl alcohol. The specimens were embedded in Epon (5) or in Araldite (6). The skin was cut on an LKB ultramicrotome ("Ultratome") with glass knives. All sections were stained with a saturated water solution of uranyl acetate at 70° C for 20–30 minutes according to Brody (7, 8). In some preparations 0.1 per cent phosphotungstic acid was added to all stages of ethyl alcohol during the dehydration of the specimens (9). The sections were examined in RCA EMU-3A and 3D electron microscopes.

RESULTS

In the cells of the basal and intermediate layers a keratin pattern is seen (1, 7, 10). It consists of longitudinally, obliquely and transversely cut less opaque filaments embedded in an opaque, interfilamentous material and arranged in fibrils. This pattern appears more or less distinct in adjacent cells and in different part of the same cell (Figs. 1 and 2).

In some of the cells in the *basal layer* the keratin fibrils may be closely packed in the whole cell. In this case the opaque, interfilamentous material is also observed between the filaments of adjacent fibrils (Fig. 1). In other cells, however, the fibrils are separated by a less opaque, non-fibriller substance (Fig. 1). This substance occurs irregularly between the fibrils. Usually it appears rather sparsely, but occasionally it may be seen in great abundance.

In the *intermediate layer* some of the cells exhibit a dense cytoplasm. Throughout the cells are observed closely packed fibrils. In these cells the opaque, interfilamentous material is also seen between the filaments of adjacent fibrils (Fig. 2). In a number of cells, however, a less opaque, non-fibrillar substance or electron-optically empty spaces are discerned between the fibrils (Figs. 2 and 3). Usually these areas are small, but occasionally cells are observed with large electron-optically empty regions separating a rather small number of fibrils (Fig. 3).

DISCUSSION

In the *basal layer* two types of cells are seen: (a) in one class of cell the longitudinal two-component system, constituting the keratin pattern (7), completely fills the cytoplasm of the cell; (b) in the other type of cell a less opaque, non-fibrillar, homogeneous substance is interspersed between keratin fibrils.

In the *intermediate layer* two types of cell are also seen. One cell type is characterized by closely packed keratin fibrils throughout the cytoplasm. No electron-optically empty spaces or areas exhibiting a less opaque, non-fibrillar, homogeneous substance are visible. It is reasonable to assume that these cells correspond to cells of the basal layer, mentioned above under (a). At the displacement of these cells from the basal to the intermediate layer they have from an electron-

* From the Department of Dermatology, Karolinska Sjukhuset, and the Department of Anatomy, Karolinska Institutet, Stockholm, Sweden.

Received for publication June 24, 1963.

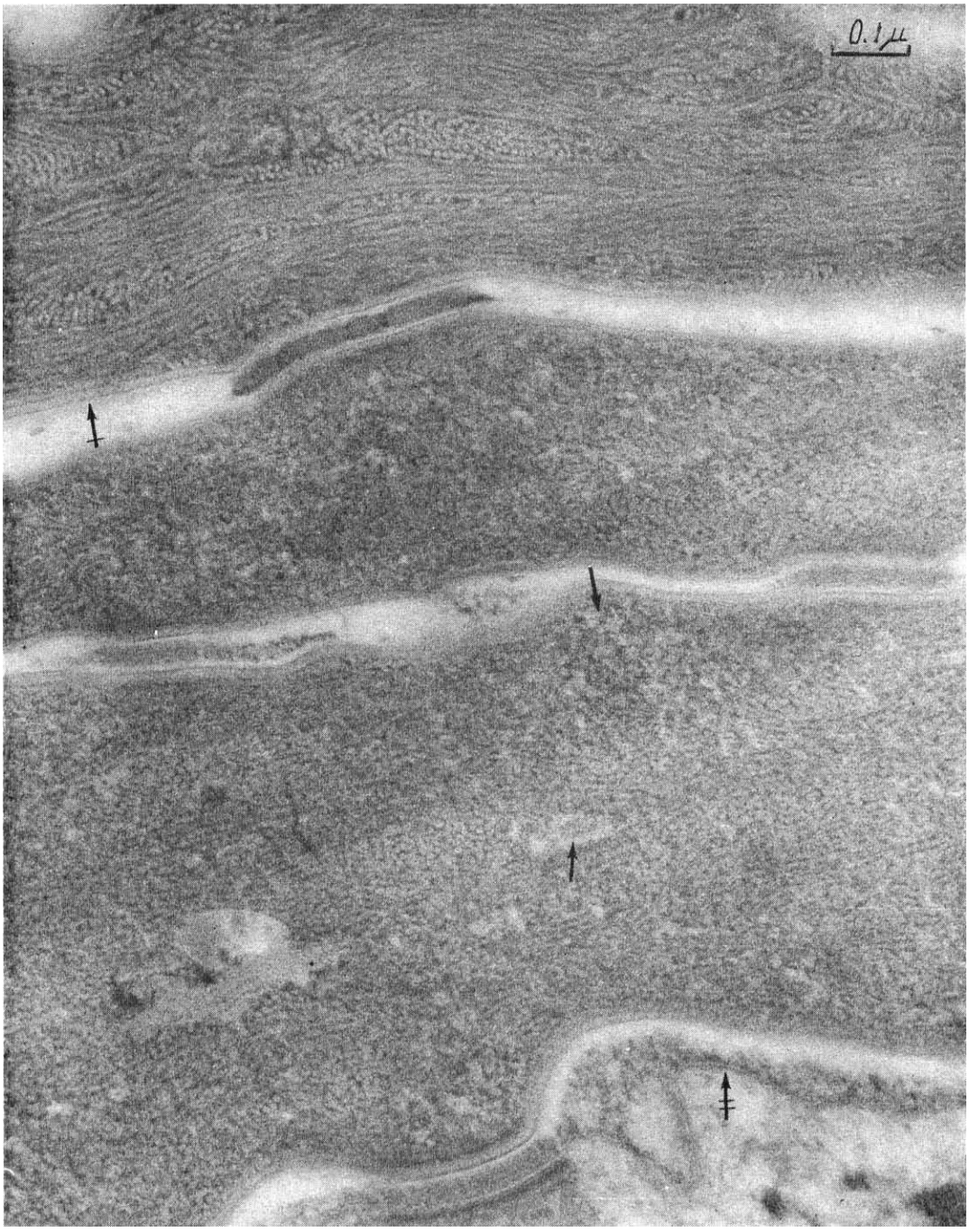

FIG. 1. Parts of cells in the basal layer of the stratum corneum. In the upper cell the keratin pattern is distinctly visible. The keratin fibrils are closely packed throughout the cell. The opaque, interfilamentous material is observed also between the filaments of adjacent fibrils. In the lower cells a keratin pattern is discerned, but appears less distinctly. Areas exhibiting a less opaque, non-fibrillar substance occur irregularly scattered between the keratin fibrils (→). The dense layer at the surface of the cells—about 140 Å in thickness—shows a distinct triple-layered pattern (+→). In the lower, right corner part of a stratum intermedium cell. The plasma membrane—about 80 Å in thickness—also displays a triple-layered pattern (‡→) (× 110,000).

FIG. 2. Parts of cells in the intermebiate layer of the stratum corneum. Two of the cells exhibit a dense cytoplasm with closely packed fibrils in the whole cytoplasm. In the other cells electron-optically empty areas are discerned to a varying extent between the fibrils. Closely occurring intercellular discs of the desmosomes (de). Intercellular substance is occasionally observed (→) (× 68,000).

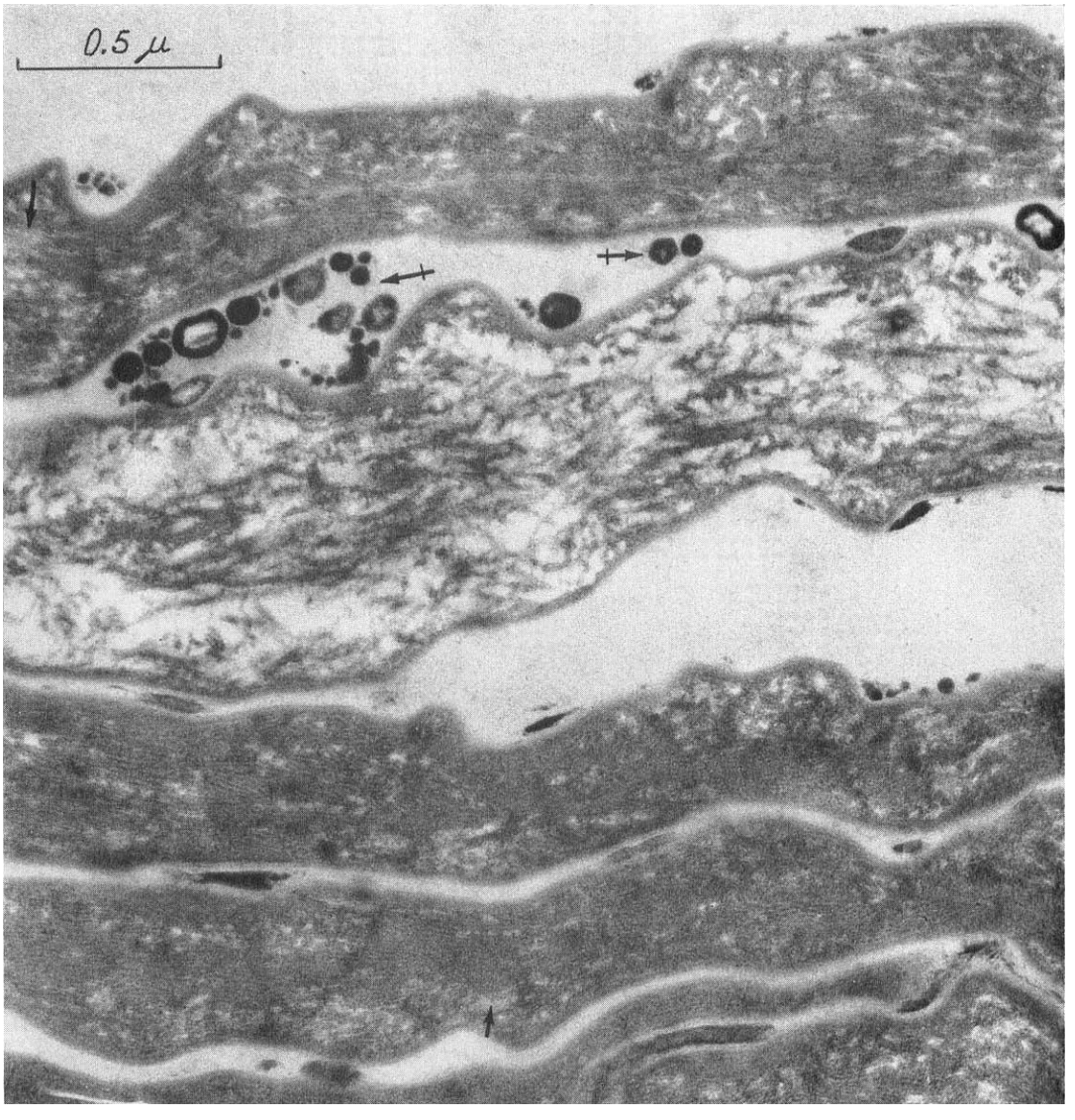

FIG. 3. Parts of cells in the intermediate layer of the stratum corneum. In one of the cells large electron-optically empty areas separate a rather small number of fibrils. In the other cells smaller electron-optically empty spaces or regions exhibiting a less opaque, non-fibrillar substance (⟶) are discerned between the fibrils. In the intercellular space components of various shape and opacity are seen (⟷) (× 54,000).

microscopic point of view only undergone a change with respect to an increased affinity of the keratin fibrils to uranyl acetate (1).

The second cell type of the intermediate layer exhibits a minor portion of a less opaque, non-fibrillar, homogeneous substance and electron-optically empty areas. It is tentatively suggested that these cells derive from those of the basal layer mentioned above under (b), where a non-fibrillar, homogeneous substance is present between the fibrils.

SUMMARY

An electron-microscopic study of the basal and intermediate layers of the stratum corneum in normal human epidermis has been carried out.

In both layers, cells can be differentiated on the basis of the absence or presence of non-fibrillar cytoplasmic areas. Parts of these non-fibrillar regions show a different electron-microscopic picture in the two layers. It is tentatively suggested that the different cell types of the intermediate layers are derived from corresponding cells in the subjacent layer.

REFERENCES

1. BRODY, I.: The ultrastructure of the horny layer in normal and psoriatic epidermis as revealed by electron microscopy. J. Invest. Derm., **39:** 519, 1962.
2. PALADE, G. E.: A study of fixation for electron microscopy. J. Exp. Med., **95:** 285, 1952.
3. SJÖSTRAND, F. S.: The ultrastructure of the outer segments of rods and cones of the eye as revealed by the electron microscope. J. Cell. Comp. Physiol., **42:** 15, 1953.
4. SJÖSTRAND, F. S.: The ultrastructure of cells as revealed by the electron microscope. Int. Rev. Cytol., **5:** 455, 1956.
5. LUFT, J.: Improvements in epoxy resin embedding methods. J. Biophys. Biochem. Cytol., **9:** 409, 1961.
6. GLAUERT, A. M., ROGERS, G. E. AND GLAUERT, R. H.: A new embedding medium for electron microscopy. Nature (London), **178:** 803, 1956.
7. BRODY, I.: The keratinization of epidermal cells of normal guinea pig skin as revealed by electron microscopy. J. Ultrastruct. Res., **2:** 482, 1959.
8. BRODY, I.: Different staining methods for the electron-microscopic elucidation of the tonofibrillar differentiation in normal epidermis. Conference on "The Biology of Keratinization" at Lake Arrowhead, California, June, 1963.
9. BRODY, I.: An ultrastructural study on the role of the keratohyalin granules in the keratinization process. J. Ultrastruct. Res., **3:** 84, 1959.
10. BRODY, I.: The ultrastructure of the tonofibrils in the keratinization process of normal human epidermis. J. Ultrastruct. Res., **4:** 264, 1960.